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(54) Title: COMPOSITION FOR PREVENTING OR TREATING MASTITIS OF DAIRY CATTLE

(57) Abstract: A composition comprising 0.1 to 20 wt% of chitosan-chitooligosaccharide and 0.1 to 10 wt% of bromelain as active ingredients is useful for decreasing the number of somatic cells in raw milk and preventing or treating mastitis.

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# COMPOSITION FOR PREVENTING OR TREATING MASTITIS OF DAIRY CATTLE

## Field of the Invention

The present invention relates to a composition for preventing or treating mastitis of dairy cattle.

## Background of the Invention

Mastitis is one of the most serious diseases that afflict dairy cows and caused by various pathogenic microorganisms e.g., *Straphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae,* and *Streptococcus uberis*. Infected cows suffer from inflamed mammary gland and produce less milk, and, further, the milk produced is of poor quality, containing an increased number of somatic cells such as neutrophils. Thus, the number of somatic cells in milk can be used as a measure of the health condition of dairy cattle and the quality of milk.

Conventionally, various antibiotics and other drugs have been used for treating mastitis. However, a long-term treatment with such drugs induces drug-resistant bacteria thereby making the mastitis chronic and incurable. Accordingly, an improved drug effective for treating or preventing mastitis has been desired.

## Summary of the Invention

It is, therefore, the object of the present invention to provide a composition effectively preventing or treating mastitis, thereby decreasing the number of somatic cells in raw milk.

## Detailed Description of the Invention

In accordance with the object, the present invention provides a

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composition for decreasing somatic cells in raw milk and effectively preventing or treating mastitis, comprising 0.1 to 20 wt % of chitosan-chitooligosaccharide and 0.1 to 10 wt% of bromelain as active ingredients. The inventive composition may further comprise 0.01 to 10 wt% of licorice, 0.5 to 30 wt% of Zn or a salt thereof and/or 10 to 60 wt% of a fermented culture.

Chitosan-chitooligosaccharide employed in the present invention is as a form of chitosan which shows a broad antibiotic effect capable of lowering the number of somatic cells. The ratio of chitosan to chitooligosaccharide is preferably 1: 0.5 to 1: 0.75.

Generally, chitooligosaccharide is prepared by enzymatically disintegrating chitin or chitosan into acetylglucosamine or glucosamine unit having a molecular weight of less than 3,000 dalton and is water-soluble. The chitosan employed in the present invention promotes the differentiation of immunocytes, and especially increases the number of polymorphonuclear leukocyte (PMN), macrophage (M) and natural killer cell (NK) to enhance the antibiotic effects of PMN plus M and NK plus M as well as the inhibitory effect against other etiological factors.

Further, the composition of the present invention comprises bromelain which is a protease having anti-inflammatory activity. Bromelain employed in the present invention is optionally coated with enteric coating agents soluble only in the intestine. Bromelain is absorbed through the intestine, but it is unstable in the highly acidic environment of the stomach of a ruminant animal such as dairy cattle. Accordingly, the coated bromelain is designed to be stable in such gastric acid environment and enhance the absorption in the intestine.

Bromelain employed in the present invention may be coated first with a 1st aqueous coating agent at a low temperature, and then with a 2nd coating agent, for an increased stability in the stomach and a controlled release in the intestine. The 1st aqueous coating agent may be sodium alginate, alginic acid, polymethylmetacrylate, e.g., Eudragait (L30D, LS30D) and Kollicoat MP (BASF), wheat protein, soybean protein, methylcellulose (MC), hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), e.g., pharma coat and aqua coat, gums such as Guar gum, Locust bean gum, Xanthan

gum, Gellan gum and Arabia gum or a mixture thereof. The aqueous coating agents are water-soluble or water-dispersible and can be easily employed by using water as a solvent, avoiding the use of toxic organic solvents. Among the above, sodium alginate is preferred.

The 2nd coating agent may be an enteric coating agent; a swell coating agent such as carbopol; and Arabia gum; or other release-control coating agents, and it is preferably a corn extract protein or an artificially processed material thereof, e.g., Zein-DP and prolamin; sodium alginate; alginic acid; polymethylmetacrylate, e.g., Eudragait (L30D, LS30D) and Kollicoat MP (BASF); shellac; hydroxypropylmethylcellulosephtalate (HPMCP); hydroxypropylmethylcellulose (HPMC); hydroxypropylmethylcelluloseacetatesuccinate (HPMCAS); carboxymethylcellulose (CMC); hydroxypropylcellulose (HPC); celluloseacetatephtalate (CAP); ethylcellulose (EC); methylcellulose (MC); soybean protein; wheat protein; chitin; chitinsan; agar; carrageenan; pectin; carbopol; gums such as Guar gum, Locust bean gum, Xanthan gum, Gellan gum and Arabia gum; or a mixture thereof, more preferably, corn extract protein, hydroxypropylmethylcellulosephtalate (HPMCP) or shellac.

Further, the composition of the present invention may further comprise 0.01 to 10 wt% of licorice, which functions to mitigate inflammation.

The composition of the present invention may still further comprise 0.5 to 30 wt% of Zn or a salt thereof. Preferably, the zinc or its salt is in the form of a chelate with methionine, acetate or protenate for improving the absorption rate thereof. A Zn compound such as zinc oxide and zinc sulfate and an organozinc such as zinc methionine, zinc acetate and zinc protenate are preferred.

Moreover, the composition of the present invention may further comprise 10 to 60 wt% of a fermented culture, which is a dried culture medium containing microorganisms in a viable form. The microorganisms employed in the present invention may be *Aspergillus oryzae, Aspergillus niger, Aspergillus terreus, Saccharomyces cerevisiae* or *Bacillus subtilis*, and, *Aspergillus oryzae* which is known to enhance milk production, is preferred.

In addition, the composition of the present invention may comprise

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formulating additives such as a solution adjuvant, a moisturizing agent, a diluent and the like, if necessary. Specifically, the solution adjuvant may be at least one selected from polyethyleneglycols, glycerin fatty acids, sorbitan fatty acid esters, propyleneglycol, glycerin, triethylcitrate, triacetin, cetyl alcohol or stearyl alcohol, and used in the amount of 0.5 to 50 wt% based on the weight of the total coated granules. The moisturizing agent may be sorbitol, mannitol, xylitol or inositol, and the diluent may be corn starch, wheat shorts, dextrose, defatted rice bran or soybean meal.

The composition of the present invention may be prepared by a process comprising the following steps of:

1) coating bromelain;
2) mixing active ingredients;
3) adding optional ingredients with the mixture obtained in step (2);
4) adding a carrier to the mixture obtained in step (3); and
5) pelletizing the mixture obtained in step (4).

In step (1), bromelain may be coated in a flowing layer assembly apparatus. A preferable embodiment of the present invention employs an aqueous solution of sodium alginate as the 1st coating agent and a coating mixture of Zein-DP and ethanol as the 2nd coating agent.

In step (2), 0.1 to 10 wt% of the coated bromelain is mixed with 0.1 to 10 wt% of chitosan-chitooligosaccharide, using a ribbon mixer at room temperature.

In step (3), 0.5 to 30 wt% of zinc or a salt thereof, 0.01 to 10 wt% of licorice and/or 10 to 60 wt% of fermented culture are added to the mixture obtained in step (2) using a mixer at room temperature.

In step (4), a carrier is mixed with the mixture obtained in step (3) using a ribbon mixer. Preferably, the carrier may be alfalfa, corn starch, gluten, wheat four or molasses.

In step (5), the mixture obtained in step (4) is pelletized, preferably at a temperature of about 121 ℃.

The composition of the present invention may be administered to dairy cows by way of admixing with feedstuff, or formulated by mixing with pharmaceutically acceptable carriers or additives for oral administration or

injection. The formulation for oral administration may be in various forms e.g., powder, granule, tablet, capsule, solution such as drench, emulsion, and suspension. The inventive composition for injection may comprise distilled water, ethyloleinate, ethanol, propyleneglycol or glycerine as a pharmaceutically acceptable carrier; ascorbic acid, sodium bisulfite, sodium pyrosulfite or tocopherol as an antioxidant; and phenylmercury nitrate, thimerosal, benzalkonium chloride, phenol, cresol, paraoxy methylbenzoate or benzyl alcohol as a preservative.

The composition may further comprise conventional vitamins, saccharides such as glucose and lactose, starch, wheat shorts, vermiculite or various feed powders and liquid enzymes for animals in an acceptable amount.

The daily dosage of the active ingredient of chitosan-chitooligosaccharide ranges from 20 to 4,000 mg/animal in case of feedstuff additive pellet, and 1,000 to 2,000 g/ton of feedstuff in case of the powder or the granule. However, it should be understood that the amount of the active ingredient actually administered should be determined in light of various relevant factors including the condition of the dairy cows to be treated, and, therefore, the dosage suggested above should not be construed to limit the scope of the invention in any way.

The composition of the present invention can be used for effective prevention or treatment of mastitis, leading to a marked decrease in the number of somatic cells in raw milk, as well as an increased milk productivity.

The following Examples are intended to further illustrate the present invention only, and are not intended to limit the scope of the invention.

Example 1: The effect of chitosan-chitooligosaccharide on the death of causative pathogens of mastitis

The minimum inhibitory concentrations (MIC) of chitosan-chitooligosaccharide on the causative bacteria of mastitis, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* (Microbiology Laboratory of College of Veterinary

Medicine, Seoul National University), were measured as follows.

Each species of bacteria was cultured in TSB medium (tryptic soy broth; bacto tryptone 17 g, bacto soytone 3 g, bacto dextrose 2.5 g, sodium chloride 5 g and dipotassium phosphate), the number of cells was counted, and diluted in TSB to $5 \times 10^4$ cells/100$\mu\ell$, and inoculated into a titration. 0.1, 0.5, 1, 3, 5, 6, 7, 8, 9, 10, and 15 mg/l of chitosan-chitooligosaccharide (1:0.75, EZBio, Inc) were respectively added thereto, cultured at 25℃ for 24 hours, 10 $\mu\ell$ (5 mg/mℓ) of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) and 10x phosphate buffer (PBS pH 7.0) were added thereto, and the extent of color developed was measured with a micro plate reader at 570nm. The results are shown in Table 1.

[Table 1]

| Concentration (mg/l) | Staphylococcus aureus | Streptococcus agalactiae | Streptococcus dysgalactiae | Streptococcus uberis |
|---|---|---|---|---|
| 0.1 | ++ | ++ | ++ | ++ |
| 0.5 | ++ | ++ | ++ | ++ |
| 1 | ++ | ++ | ++ | ++ |
| 3 | ++ | ++ | ++ | ++ |
| 5 | + | ++ | + | ++ |
| 6 | + | + | + | ++ |
| 7 | + | + | + | + |
| 8 | + | + | + | + |
| 9 | + | + | - | + |
| 10 | - | - | - | - |
| 15 | - | - | - | - |
| MIC | 10 mg/l | 10 mg/l | 9 mg/l | 10 mg/l |

Table 1 shows that MIC of chitosan-chitooligosaccharide is 9 to 10 mg/l for each species of bacteria.

Example 2: The controlling effect of the combined use of chitosan-chitooligosaccharide and bromelain on the causative pathogens of mastitis

In order to check the controlling effect of the combination of chitosan-chitooligosaccharide and bromelain on the causative pathogens of mastitis, the MIC of the combination was measured by the procedure of Example 1. 1 mg/l of 1000 CDU/mg bromelain (B) (Pineapple meal, Hong Mao Biochemical Co., Ltd., Taiwan) was combined with 0.1, 0.5, 1, 3, 5, 6, 7, 8, 9, 10, or 15 mg/l of chitosan-chitooligosaccharide (C). CDU (Casein Digestion Unit), the amount of bromelain required for isolating 1 $\mu g$ of tyrosine from casein at 37℃ for 1 minute, represents the enzymatic activity of bromelain, and 1000 CDU means that 1 mg of bromelain isolates 1 mg of tyrosine. The results are shown in Table 2.

[Table 2]

| Concentration (C+B) (mg/l) | Staphylococcus aureus | Streptococcus agalactiae | Streptococcus dysgalactiae | Streptococcus uberis |
|---|---|---|---|---|
| 0.1+1 | ++ | ++ | ++ | ++ |
| 0.5+1 | ++ | ++ | ++ | ++ |
| 1+1 | ++ | ++ | ++ | ++ |
| 2+1 | ++ | ++ | + | ++ |
| 3+1 | + | + | + | + |
| 5+1 | + | + | + | + |
| 6+1 | + | + | + | + |
| 7+1 | + | + | + | + |
| 8+1 | + | + | - | - |
| 9+1 | - | - | - | - |
| MIC | 9 mg/l | 9 mg/l | 8 mg/l | 8 mg/l |

Table 2 shows that the MIC of the chitosan-chitooligosaccharide and

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bromelain (C+B) combination is 8 to 9 mg/l for each species of bacteria, which is lower than the MIC of chitosan-chitooligosaccharide alone.

Example 3: The effect of chitosan-chitooligosaccharide on the number of somatic cells in raw milk

In order to identify the optimal concentration of chitosan-chitooligosaccharide for lowering somatic cells in raw milk, 5, 7.5 and 10 mg daily doses of chitosan-chitooligosaccharide dissolved in water were orally administered to respective experimental groups by using a PET bottle, while a control group was not given chitosan-chitooligosaccharide. Each group consisted of 10 dairy cows, and milking was carried out for 8 weeks. The change of the number of somatic cells in raw milk was measured with Fossomatic 300, wherein the somatic cells were coated with fluorescent ethydium bromide and spread on a disk, and the number of the somatic cells was measured with a halogen lamp. The mean value of the results that obtained is shown in Table 3.

[Table 3]

| Somatic cell counts ($10^4$/ml) Time (week) | chitosan-chitooligosaccharide (dosage/day) | | | |
|---|---|---|---|---|
| | 0 mg | 5 mg | 7.5 mg | 10 mg |
| 0 | 67.4 | 68.1 | 67.5 | 67.2 |
| 1 | 62.0 | 51.9 | 49.0 | 48.5 |
| 2 | 63.9 | 49.8 | 38.7 | 36.1 |
| 3 | 68.1 | 31.1 | 29.1 | 27.5 |
| 4 | 66.2 | 30.8 | 25.3 | 23.8 |
| 5 | 67.1 | 32.7 | 23.2 | 24.5 |
| 6 | 62.0 | 29.9 | 24.1 | 23.5 |
| 7 | 60.1 | 32.1 | 22.9 | 23.0 |
| 8 | 64.2 | 30.1 | 22.0 | 22.7 |

Table 3 demonstrates that the number of somatic cells in the raw milk taken from the experimental groups is markedly lower than that of the control group. Specifically, 5, 7.5 and 10 mg groups showed 53.1%, 65.7% and 64.6% decrease in the number of somatic cells as compared to the control group, respectively. Accordingly, an optimal concentration of chitosan-chitooligosaccharide for lowering the number of somatic cells in raw milk was determined to be 7.5 mg/day.

Example 4: The effect of licorice in lowering the number of somatic cells

In order to measure the effect of licorice in lowering the somatic cells in raw milk, 100, 200 and 300 mg daily doses of licorice containing 7% of glycyrrhizine as an active ingredient (Daepyung, Inc.) were orally administered to respective experimental groups, while a control group was not given licorice. Each group consisted of 10 dairy cows, and milking was carried out for 6 weeks. The change of the number of somatic cells in raw milk was measured by the method of Example 3. The results are shown in Table 4.

[Table 4]

| Somatic cell counts ($10^4$/ml) / Time (week) | Licorice (dosage/day) | | | |
|---|---|---|---|---|
| | 0 mg | 100 mg | 200 mg | 300 mg |
| 0 | 80.2 | 80.1 | 81.0 | 80.5 |
| 1 | 81.2 | 69.4 | 69.9 | 70.1 |
| 2 | 81.4 | 51.2 | 51.4 | 49.2 |
| 3 | 80.8 | 40.5. | 40.3 | 38.7 |
| 4 | 79.8 | 35.1 | 38.7 | 35.1 |
| 5 | 80.4 | 30.8 | 35.1 | 34.2 |
| 6 | 81.0 | 30.0 | 34.4 | 35.8 |

As shown in Table 4, the experimental groups show lower somatic cell number than the control group.

Example 5: The effect of zinc in lowering the number of somatic cells

In order to examine the effect of zinc in lowering the number of somatic cells in raw milk, 100 and 200 mg daily doses of zinc in the form of zinc-methionine (Samjo, Inc.) dissolved in water were orally administered to respective experimental groups by using a catheter bottle, while a control group was not given zinc methionine. Each group consisted of 20 dairy cows, and milking was carried out for 8 weeks. The change in the number of somatic cells in raw milk was measured by the method of Example 3. The results are shown in Table 5.

[Table 5]

| Somatic cell counts ($10^4$/ml) Time (week) | Zinc methionine (dosage/day) | | |
|---|---|---|---|
| | 0 mg | 100 mg | 200 mg |
| 0 | 56.7 | 54.1 | 55.2 |
| 1 | 55.1 | 40.1 | 39.7 |
| 2 | 54.1 | 35.2 | 34.9 |
| 3 | 56.1 | 30.9 | 29.4 |
| 4 | 55.1 | 28.1 | 27.9 |
| 5 | 57.1 | 30.6 | 28.4 |
| 6 | 54.5 | 27.3 | 26.8 |
| 7 | 55.3 | 28.2 | 29.8 |
| 8 | 57.1 | 27.9 | 28.1 |

As shown in Table 5, the experimental groups show lower somatic cell numbers than the control group. Specifically, the 100 and 200 mg groups show somatic cell numbers which were 51.1% and 50.8% lower than that of the

control group, respectively. Accordingly, an optimal concentration of zinc for lowering the number of somatic cells in raw milk was determined to be 100 mg/day.

Example 6: The stability of the coated bromelain in gastric acid

The pH in stomach of dairy cattle is 1 to 2, and digestion takes about 6 hours. As the optimal pH for bromelain activity is 4.5 to 5.0, bromelain of the present invention was double-coated for the protection thereof in such a gastric environment as follows.

1000 CDU of bromelain (Pineapple meal Hong Mao Biochemical Co., Ltd., Taiwan) was coated first by spraying an aqueous solution of sodium alginate, and then coated with a solution containing Zein-DP in 80% ethanol, using a flowing layer assembly apparatus.

An artificial gastric solution of pH 1.2 was prepared in accordance with the standard manual of United States Pharmacopeia (USP). Coated and uncoated bromelains of 1000 CDU were each soaked in 0.1 l of the artificial gastric solution for 6 hours, and the CDU of each bromelain was determined by measuring the amount of tyrosine discharged while adding each bromelain to an aqueous casein solution by 1mg at a time.

Uncoated and coated bromelains showed 300 and 900 CDU, respectively. The double-coated bromelain according to the present invention was thus confirmed to be stable and maintain its enzymatic activity in the gastric acid.

Example 7: The effect of the coated bromelain in lowering the number of somatic cells

In order to measure the effect of coated bromelain in lowering the number of somatic cells in raw milk, 3, 6, and 9 mg daily doses of coated bromelain dissolved in water was orally administered to respective experimental groups by using a catheter bottle, while a control group was not given the coated bromelain. Each group consisted of 10 dairy cows, and

milking was carried out for 6 weeks. The change of the number of somatic cells in raw milk was measured by the method of Example 3. The results are shown in Table 6.

[Table 6]

| Somatic cell counts ($10^4$/ml) / Time (week) | Coated Bromelain (dosage/day) | | | |
|---|---|---|---|---|
| | 0 mg | 3 mg | 6 mg | 9 mg |
| 0 | 84.4 | 85.1 | 86.0 | 84.5 |
| 1 | 85.2 | 70.4 | 65.8 | 60.1 |
| 2 | 86.7 | 61.2 | 47.1 | 42.2 |
| 3 | 84.1 | 42.5 | 35.1 | 38.7 |
| 4 | 85.5 | 40.1 | 38.7 | 30.1 |
| 5 | 82.0 | 41.8 | 30.1 | 31.2 |
| 6 | 83.4 | 40.7 | 31.4 | 30.8 |

As shown in Table 6, the experimental groups showed markedly lower somatic cell numbers in raw milk than the control group. Specifically, the 3, 6, and 9 mg groups showed somatic cell numbers which are 51.2%, 62.35% and 63.07% lower than that of the control group, respectively. Accordingly, an optimal concentration of coated bromelain for lowering the number of somatic cells in raw milk was determined to be 9 mg/day.

Example 8: The effect of the combination of chitosan-chitooligosaccharide, zinc methionine and coated bromelain in lowering the number of somatic cells

Aqueous solutions of 7.5 mg/l containing chitosan-chitooligosaccharide, 100 mg/l of zinc methionine and 9 mg/l of coated bromelain, respectively, were prepared and mixed to obtain formulation A which contained chitosan-chitooligosaccharide and zinc methionine, as well as formulation B which contained chitosan-chitooligosaccharide, zinc methionine and coated bromelain. The formulations were orally administered to respective experimental groups,

while only water was fed to a control group. Each group consisted of 30 dairy cows, and milking was carried out for 8 weeks. The change in the number of somatic cells in raw milk was measured by the method of Example 3 and the results are shown in Table 7.

[Table 7]

| Somatic cell counts ($10^4$/ml) / Time (week) | Control | Formulation A | Formulation B |
|---|---|---|---|
| 0 | 50.1 | 51.2 | 50.9 |
| 1 | 49.8 | 40.1 | 40.6 |
| 2 | 51.1 | 32.7 | 31.0 |
| 3 | 50.0 | 27.4 | 22.1 |
| 4 | 48.9 | 19.7 | 16.7 |
| 5 | 49.7 | 20.1 | 15.4 |
| 6 | 51.1 | 18.5 | 17.0 |
| 7 | 49.1 | 19.1 | 16.1 |
| 8 | 49.7 | 18.7 | 15.1 |

The number of somatic cells for the control group was fluctuated a little depending on the environmental change. As indicated in Table 7, the experimental groups administered with the composition of the present invention showed a marked decrease in the number of somatic cells in raw milk as compared with the control group. Specifically, formulation A group shows a 62.4% decrease in the number of somatic cell as compared to the control group, and formulation B group, a 69.6% decrease, while the grade of the raw milk was improved from the 2nd or 3rd to 1st. Also, formulation B was found to be superior to formulation A.

Example 9: The effect of fermented culture on increasing milk production

The effect of the culture of *Aspergillus oryzae* (Genebiotech, Inc.) in lowering the number of somatic cells and increasing milk production was tested. *Aspergillus oryzae* was cultured by a solid fermentation method (Raper, K.B. et al., The genus *Aspergillus*, R.E. Krieger Publishing company, Huntington, NY, USA, 1965). 200 g of the dried culture was added to formulation B of Example 7 to obtain formulation C. Formulation B and formulation C were orally administered to respective experimental groups. Each group consisted of 40 dairy cows, and milking was carried out for 5 weeks. The change in the number of somatic cells in raw milk is shown in Table 8.

[Table 8]

| | Somatic cell counts ($10^4$/ml) | Milk production (kg/day/cow) |
|---|---|---|
| Control | 49.8 | 29.7 |
| Formulation B | 15.7 (68.4% Decrease) | 31.8 (7.1% Increase) |
| Formulation C | 16.0 (67.9% Decrease) | 33.1 (11.4% In crease) |

As shown in Table 8, the experimental groups shows about 68% lower number of somatic cells than the control group, while the formulation B and C groups give 7.1 % and 11.1% increases in milk production, respectively, as compared to the control group.

<u>Example 10: The effect of the combination of chitosan-chitooligosaccharide, zinc methionine, coated bromelain, licorice and fermented culture in lowering the number of somatic cells</u>

Formulation D containing 11.4 wt% of chitosan-chitooligosaccharide, 1 wt% of coated bromelain, 10 wt% of zinc methionine, 10 wt% of licorice and 20 wt% of fermented culture was prepared. The formulation was orally administered to experimental groups, while only water was fed to control groups. Experimental groups and control groups were respectively consisted of 74 and 27 dairy cows collected from 5 farms as shown in Table 9 and

milking was carried out every 2 weeks for 10 weeks. The change in the number of somatic cells in raw milk was measured by the method of Example 3 and the results are shown in Table 9.

[Table 9]

| Farm | Group | No. of Cows | Somatic cell counts (Mean × $10^4$/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 week | 2 week | 4 week | 6 week | 8 week | 10 week |
| A | Exp | 19 | 61.4 | 47.0 | 34.0 | 38.9 | 36.4 | 37.2 |
| | Con | 11 | 13.7 | 28.4 | 12.2 | 23.1 | 20.1 | 29.3 |
| B | Exp | 4 | 6.4 | 12.1 | 18.9 | 8.9 | 10.7 | 12.5 |
| | Con | 16 | 11.6 | 15.4 | 18.2 | 15.5 | 16.4 | 16.8 |
| C | Exp | 17 | 66.5 | 56.6 | 43.2 | 28.1 | 29.3 | 28.9 |
| D | Exp | 10 | 17.4 | 15.4 | 9.1 | 12.7 | 9.4 | 13.0 |
| E | Exp | 24 | 20.7 | 17.2 | 18.7 | 21.8 | 17.9 | 21.6 |
| Total | Exp | 74 | 34.5 (100%) | 29.7 (86.0%) | 24.8 (71.8) | 22.1 (64.0%) | 21.2 (61.5%) | 22.5 (65.2%) |
| | Con | 27 | 12.7 (100%) | 21.9 (173%) | 15.2 (119.8%) | 19.3 (152.7%) | 18.3 (143.7%) | 23.1 (181.9%) |

As indicated in Table 9, the experimental groups administered with the composition of the present invention showed a marked decrease in the number of somatic cells in raw milk as compared with the control groups. Specifically, the experimental groups shows a 14.0%, 28.2%, 36%, 38.5% and 34.8% decrease in the number of somatic cells 2, 4, 6, 8 and 10 weeks after the administration of formulation D, respectively. The number of somatic cells started to decrease 2 weeks after the administration and lowest 6 to 8 weeks after the administration.

Example 11: The effect of the combination of chitosan-chitooligosaccharide, zinc methionine, coated bromelain, licorice and fermented culture in lowering the number of somatic cells and milk productibility

Formulation D of Example 10 was orally administered to 623 dairy cows for 42 days in Corona, California, U.S.A. The daily dosage of administration was 20g/cow. Milking was carried out for 6 weeks and the change in the number of somatic cells in raw milk and the amount of produced milk are shown in Table 10.

[Table 10]

| Time (week) | 0 | 6 |
|---|---|---|
| Somatic Cell Counts ($10^4$/ml) | 57 | 34 (60% Decrease) |
| Milk Production (l/day /cow) | 51.6 | 59.0 (14% Increase) |

As shown in Table 10, the composition of the present invention induced a marked decrease in the number of somatic cells in raw milk and an increase in the amount of milk production.

While the invention has been described with respect to the above specific embodiments, it should be recognized that various modifications and changes may be made to the invention by those skilled in the art which also fall within the scope of the invention as defined by the appended claims.

**What is claimed is:**

1. A composition for preventing or treating mastitis, comprising 0.1 to 20 wt% of chitosan-chitooligosaccharide and 0.1 to 10 wt% of bromelain as active ingredients.

2. The composition of claim 1, wherein said chitosan-chitooligosaccharide is a mixture of chitosan and chitooligosaccharide, in a ratio ranging from 1: 0.5 to 1: 0.75.

3. The composition of claim 1, wherein said bromelain is coated first with an aqueous 1st coating agent and then coated with a 2nd coating agent.

4. The composition of claim 3, wherein the aqueous 1st coating agent is selected from the group consisting of sodium alginate, alginic acid, polymethylmetacrylate, wheat protein, soybean protein, methylcellulose (MC), hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), Guar gum, Locust bean gum, Xanthan gum, Gellan gum, Arabia gum and a mixture thereof.

5. The composition of claim 3, wherein the 2nd coating agent is selected from the group consisting of corn extract protein or an artificially processed material thereof, sodium alginate, alginic acid, polymethylmetacrylate, shellac, hydroxypropylmethylcellulosephtalate (HPMCP), hydroxypropylmethylcellulose (HPMC), hydroxypropylmethylcelluloseacetatesuccinate (HPMCAS), carboxymethylcellulose (CMC), hydroxypropylcellulose (HPC), celluloseacetatephtalate (CAP), ethylcellulose (EC), methylcellulose (MC), soybean protein, wheat protein, chitin, chitinsan, agar, carrageenan, pectin, carbopol, Guar gum, Locust bean gum, Xanthan gum, Gellan gum, Arabia gum and a mixture thereof.

6. The composition of claim 1, which further comprises 0.5 to 30 wt% of

zinc or a salt thereof.

7. The composition of claim 6, wherein the zinc or its salt is chelated with a compound selected from the group consisting of methionine, acetate and protenate.

8. The composition of claim 1 or 6, which further comprises 0.01 to 10 wt% of licorice.

9. The composition of claim 1 or 6, which further comprises 10 to 60 wt% of a fermented culture.

10. The composition of claim 8, which further comprises 10 to 60 wt% of a fermented culture.

11. The composition of claim 9, wherein the fermented culture is the culture of the microorganism selected from the group consisting of *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus terreus*, *Saccharomyces cerevisiae* and *Bacillus subtilis*.

12. The composition of claim 10, wherein the fermented culture is the culture of the microorganism selected from the group consisting of *Aspergillus oryzae*, *Aspergillus niger*, *Aspergillus terreus*, *Saccharomyces cerevisiae* and *Bacillus subtilis*.

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR03/00031

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC7 A61K 35/78

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC7 A61K 35/78, A 61K 31/73, C07H 15/203

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
KOREAN PATENTS AND APPLICATIONS FOR INVENTIONS SINCE 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
pubmed on line

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | JP 04-169529 A (NIPPON SODA CO., LTD.) 17 June 1992<br>see pages 1-4 | 1 |
| A | EP 0692253 A (NIPPON SODA CO., LTD. ) 17 Jan. 1996<br>see pages 1-3 | 1 |
| A | JP 57-180602 A (NIPPON SODA CO., LTD. ) 06 Nov.1982<br>see pages 1-5 | 1 |
| A | KR 1999-0044796 A (SEI WON HWA SEONG CO., LTD.) 25 June 1999<br>see the whole document | 1 |
| A | KR 1998-0008232 A (KIN, CHAN) 30 April 1998<br>see pages 1-4 | 1 |
| P, A | KR 2001-0058414 A (Lee, Dong Ku) 06 June 2001<br>see the whole document | 1 |

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
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Information on patent family members

International application No.
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| KR 2001-0058414 A | 06/07/2001 | NONE | |

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